PROMOTION OF FERTILIZATION MEMBRANE FORMATION WITH PERIODATE IN THE EGGS OF THE SEA URCHIN, HEMICENTROTUS PULCHERRIMUS

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PROMOTION OF FERTILIZATION MEMBRANE FORMATION WITH PERIODATE IN THE EGGS OF THE SEA URCHIN, *HEMICENTROTUS PULCHERRIMUS*[1)]

KENZI OSANAI[2)]

Marine Biological Station, Tohoku University, Asamushi, Aomori, 039-34 Japan

In the underripe and overripe eggs of the sea urchin, *Hemicentrotus pulcherrimus*, the irregular tight membrane formed after fertilization in ordinary sea water, but the normal fertilization membrane was elevated by the treatment with $0.4\text{–}1 \times 10^{-4}$M sodium metaperiodate sea water. Concerning the promotion of fertilization membrane formation periodate was effective in the period from one to two minutes after insemination. During the period the eggs began to elevate the fertilization membrane and completed it. These results suggest that the process of fertilization membrane formation or the breakdown or discharge of the cortical granules is promoted with periodate.

The underripe or overripe eggs of the sea urchin, *Hemicentrotus pulcherrimus*, collected at early or late season of breeding period, tend to form an abnormal or arrested membrane after fertilization. The fertilization membrane does not elevate highly or swells irregularly like a fringe. RUNNSTRÖM and KRISZAT (1950) reported that in the underripe eggs of *Psammechinus miliaris* fertilization and fertlilization membrane formation were promoted by the treatment with periodate. Therefore, it was examined whether fertilization membrane formation could be promoted also with periodate in *Hemicentrotus* eggs. In *Hemicentrotus* not only underripe eggs, but also overripe eggs formed the normal fertilization membrane in periodate sea water. It seems to be useful for studying egg ripeness to examine the action of periodate on sea urchin eggs.

MATERIAL AND METHOD

The materials were *Hemicentrotus pulcherrimus* collected at Asamushi, Aomori. The breeding season of this species is from late December to May at Asamushi. The gamates were discharged by injecting 0.5 M KCl solution into the body cavity. The eggs obtained from the sea urchins collected in January, 1976, showed irregular membrane formation in ordinary sea water. These eggs, which were abnormal in membrane formation, were used for experiments on the promotion of membrane

1) Contributions from the Marine Biological Station, Tohoku University, Aomori City, No. 424

2) 長内 健治

formation. Sodium metaperiodate ($NaIO_4$, Kanto Chemical Co.), a promoting agent, was dissolved in distilled water at 1×10^{-3}M. The aqueous solution was diluted with sea water prior to its use.

EXPERIMENT AND RESULT

1. *Fertilization membrane formation in underripe and overripe eggs.*

When the eggs obtained from the sea urchins collected in January were inseminated in ordinary sea water, fertilization rate was low and the formed fertilization membrane was tight or irregular (Figs. 1 and 2). Aged eggs placed in sea water for a long period were also regressive in membrane formation. Overripe eggs obtained at later breeding season formed the irregular membrane. The abnormalities

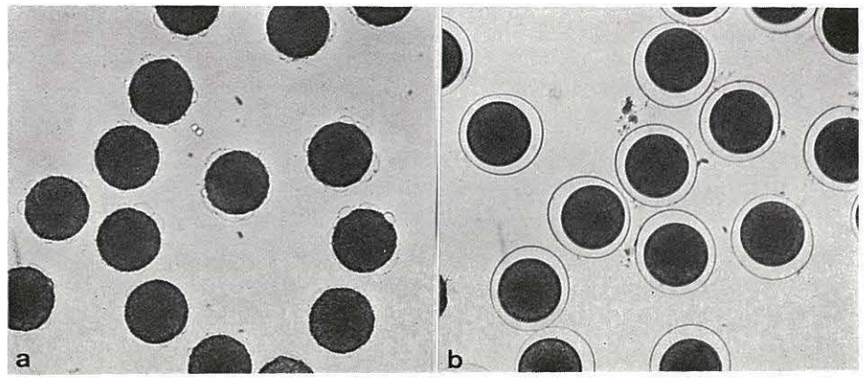

Fig. 1. Fertilization membrane formation in the underripe eggs of *Hemicentrotus pulcherrimus*. The eggs were inseminated in ordinary sea water (a) and in 5×10^{-5}M $NaIO_4$ sea water (b). About 35 minutes after insemination. × 80

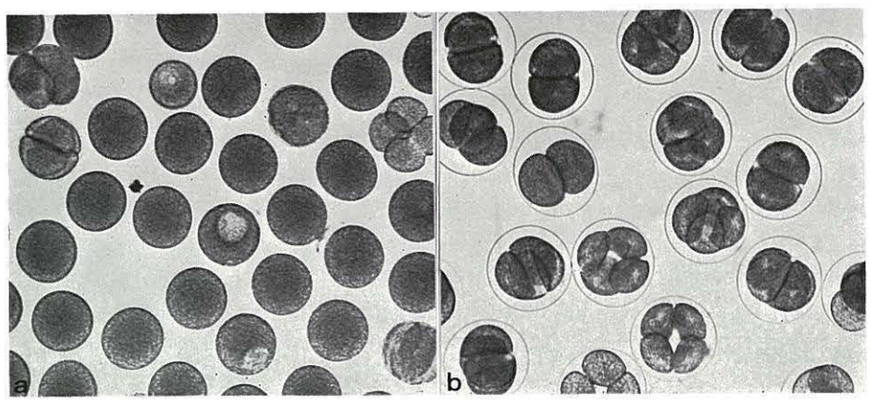

Fig. 2. Cleavage of the underripe eggs. The eggs were inseminated in ordinary sea water (a) and in 5×10^{-5} M $NaIO_4$ sea water (b). About 3 hours after insemination. × 80

of the fertilization membrane observed in these eggs were as follows; (1) partially elevated membrane on a part of the egg surface without spreading over the whole surface, (2) tight membrane adhering to the hyaline layer, (3) fringe-like membrane from which small bulges swelled out, (4) deformed membrane, which was not smooth though it was separated from the whole egg surface.

When the eggs formed the irregular membrane, the egg cells were also deformed in accordance with the shape of the membranes. The deformed eggs could not follow normal cleavage pattern.

2. *Membrane formation in periodate sea water.*

The eggs taken from batches, in which the fertilization membrane were formed abnormally, were immersed in $NaIO_4$ sea water for five minutes and then inseminated in periodate sea water in which they were allowed to develop. Fertilization membrane formation and cleavage were observed respectively one hour and 3.5 hours after insemination. The results are shown in Table 1. In the concentration from 4×10^{-5}M to 1×10^{-4}M of $NaIO_4$ membrane elevation and fertilization were remarkably promoted and the smooth membrane was highly elevated. In the eggs inseminated in 1×10^{-5}M $NaIO_4$ sea water cleavage rate was lower than that of the controlled eggs. This may be due to lowered sperm concentration, because the spermatozoa were aggregated on the precipitates of periodate which formed a deposit in sea water.

Table 1
Fertilization in $NaIO_4$ sea water

Concentration of $NaIO_4$ ($\times10^{-5}$M)	A		B	
	Membrane elevation	Cleavage	Membrane elevation	Cleavage
0	0 %	66.0%	0 %	39.0%
1	0	6.0	0	11.0
2	7.4	21.0	33.3	34.0
4	97.9	97.0	77.1	77.0
7	97.5	99.0	89.5	88.5
10	98.8	100	78.3	77.0

The eggs were placed in periodate sea water for 5 minutes and then inseminated. Fertilization membrane formation and cleavage were observed respectively 1 hour and 3.5 hours after insemination.

3. *Pretreatment with periodate.*

As mentioned above, fertilization membrane formation is promoted in the presence of periodate. If the low activity of membrane formation is attributed to the presence of an inhibiting factor as with the view of Runnström and Kriszat (1950), the factor would be removed by periodate pretreatment. The eggs were exposed to 1×10^{-4}M for various periods and then returned to normal sea water,

in which they were inseminated. In the case shown in Table 2, the controlled eggs formed the irregular fringe-like membrane and normal membrane formation was not induced by the pretreatment for less than 30 minutes. By the pretreatment for 180 minutes the percentage of the normal membrane was slightly increased. In the case shown in Table 3 membrane formation was slightly improved by the treatment for 4 minutes, but the improvement seems to be attributed to the effect of washing, because the same degree of membrane formation was observed in the eggs washed with fresh sea water (the control C_1). The membrane formation could not be promoted markedly by the periodate pretreatment.

Table 2

Fertilization membrane elevation in periodate-pretreated eggs (1)

Time of $NaIO_4$-pretreatment (min.)[a]	0	10	20	30	180
Membrane elevation (%)					
irregular	74.2	93.0	95.1	93.1	76.7
normal	0	0	0	0	20.2

a) The eggs were pretreated with 1×10^{-4}M $NaIO_4$ sea water and then inseminated in ordinary sea water. Membrane formation was counted about one hour after fertilization.

Table 3

Fertilization membrane elevation in periodate-pretreated egg (2)

Time of $NaIO_4$-pretreatment (min.)[a]	0	0.5	1	2	4	C_1[b]	C_2[c]
Membrane elevation (%)	0	0	0	8.9	10.8	9.2	82.6

a) The eggs were pretreated with 1×10^{-4}M $NaIO_4$ sea water and then inseminated ordinary sea water.
b) C_1 is the control in which the eggs were inseminated after being washed with fresh sea water for 4 minutes without periodate pretreatment.
c) C_2 is the other control. The eggs were inseminated and allowed to develop in periodate sea water.

4. *Effect of removing jelly coat.*

The intact jelly coat and dissolved jelly substance of sea urchin eggs inhibit fertilization (HAGSTRÖM 1956a, b: RUNNSTRÖM 1950). The jelly coat is known to be decomposed by periodate. If the low activity of membrane formation is due to a factor consisting the jelly coat, which can be destroyed with periodate, it should be expected that the membrane elevation may be improved by removing the jelly coat. The unfertilized eggs were exposed to HCl sea water, pH 4.3, for ten minutes. The jelly coat was not detected around the eggs. After washing with sea water, a part of the jellyless eggs was inseminated in normal sea water and the other in 5×10^{-5}M $NaIO_4$ sea water. In the jellyless eggs inseminated in sea

water the number of the partial or irregular membrane increased, but the smooth membrane was not observed. The eggs inseminated in periodate sea water formed the highly elevated smooth membrane whether the jelly coat was present or not (Table 4). This result shows that the removal of the jelly coat scarcely contributes to the membrane formation.

Table 4

Effect of jelly removal on fertelization membrane formation

Egg	Insemination medium	Membrane elevation[a] (%)		
		−	±	+
With jelly	Sea water	69.5	30.5	0
	$NaIO_4$-sea water[b]	17.8	0	88.2
Jellyless	Sea water	0.8	99.2	0
	$NaIO_4$-sea water[b]	14.7	0.4	84.9

a) − No membrane formation
± Partial or irregular membrane
\+ Highly elevated smooth membrane
b) The eggs were inseminated in 5×10^{-5}M $NaIO_4$ sea water.

5. *Sensitive period to periodate.*

As described above, the formation of the fertilization membrane was remarkably promoted in the presence of periodate, while the periodate pretreatment was not so effective. These results suggest that periodate should influence on the process of the cortical response at fertilization. In order to know the period sensitive to periodate, membrane formation was observed when the eggs inseminated in ordinary sea water were transferred to periodate sea water and when the eggs inseminated in periodate sea water were returned to oridinary sea water.

The eggs were inseminated in oridinary sea water and transferred to 5×10^{-5} M $NaIO_4$ sea water at various intervals after insemination. The membrane formation was counted more than 30 minutes after insemination. When the eggs were transferred to $NaIO_4$ sea water within one minute the smooth membrane was elevated. In the eggs transferred after more than 3 minutes the membrane elevation was scarcely observed. In the transference 2 minutes after insemination the normal membranes were scarcely observed, but the partial membranes were formed. These results suggest that periodate is effective in the process of the cortical change following one minute after insemination.

The eggs were inseminated in 5×10^{-5}M $NaIO_4$ sea water and then returned to normal sea water. The eggs palced in periodate sea water for 1.5 or 2 minutes following insemination could form the normal fertilization membranes. When the eggs were transferred from periodate to sea water within one minute, no membrane was elevated. Thus, it was ascertained that fertilization membrane formation was improved by transferring the eggs to periodate sea water within one

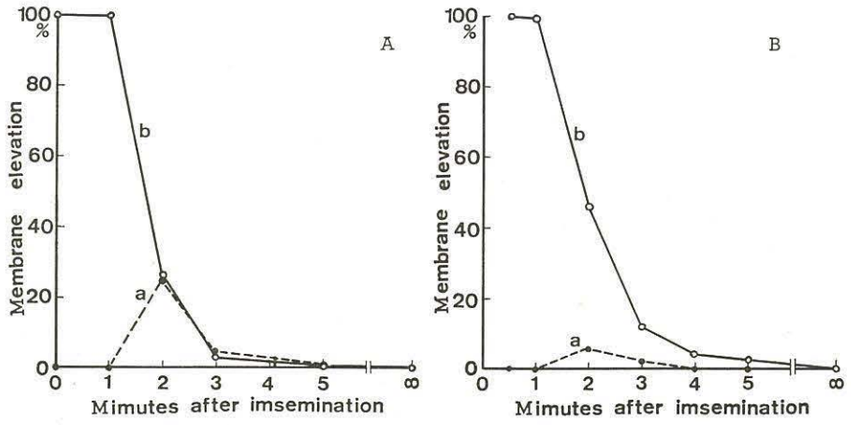

Fig. 3. Membrane formation in underripe eggs transferred to periodate sea water (5×10^{-5} M) after being inseminated in ordinary sea water. The abscissa indicates time from insemination until transference to periodate sea water. ∞ is the control placed in ordinary sea water and 0 is the other control inseminated and allowed to develop in the presence of periodate. a; partial or irregular membrane, b: highly elevated membrane.

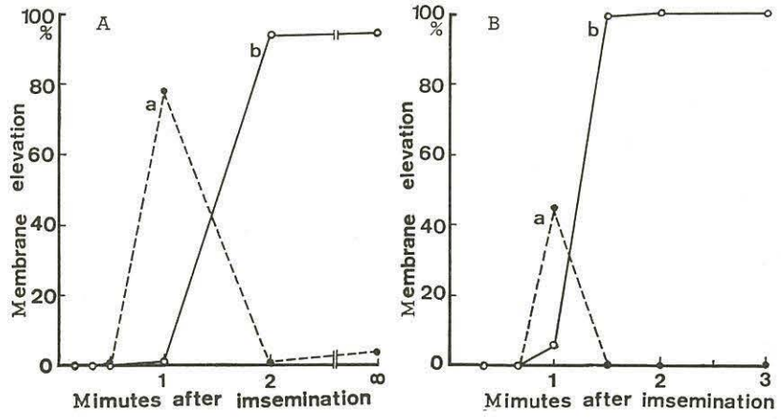

Fig. 4. Fertilization membrane formation in the underripe eggs returned to ordinary sea water after being inseminated in 5×10^{-5}M $NaIO_4$ sea water. The abscissa indicates time from insemination until transference to ordinary sea water. ∞ is the control placed in periodate sea water. a: partilly or irregular membrane, b: highly elevated smooth membrane.

minutes after insemination or by incubating them in periodate for 2 minutes following insemination. These results show that concerning the promotion of membrane formation the *Hemicentrotus* eggs are sensitive to periodate in the period from one minute to two minutes after insemination.

6. *Time cource of fertilization membrane formation in periodate sea water.*

In the eggs inseminated and allowed to develop in 5×10^{-5}M $NaIO_4$ sea water the cortical changes following fertilization were traced. At one minute after insemination the fertilization membrane began to elevate in one side of the egg surface. Many spermatozoa attached on the elevating membrane or the vitelline

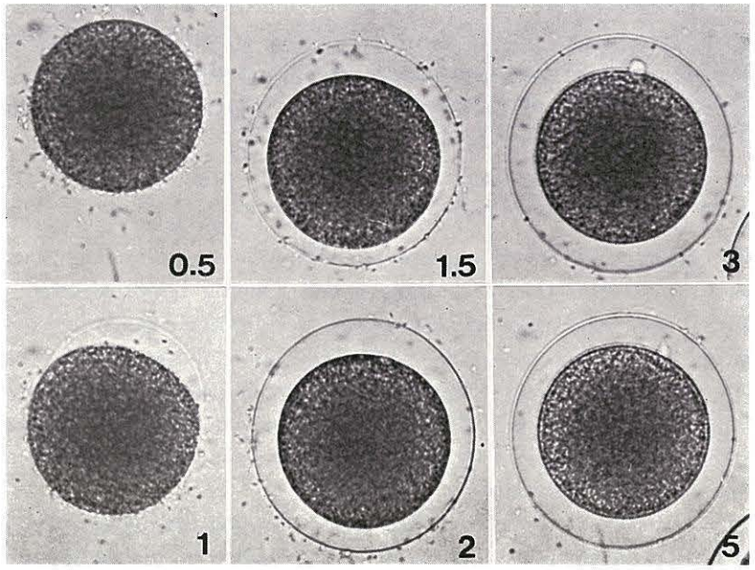

Fig. 5. Post-fertilization changes of the *Hemicentrotus* egg in periodate sea water (5×10^{-5} M). Numbers indicate minutes after insemination. × 200

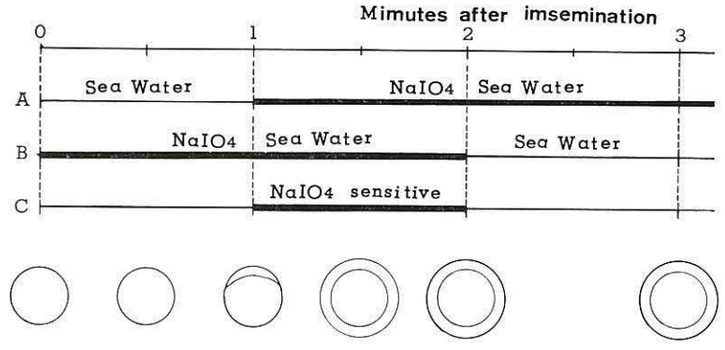

Fig. 6. Periodate sensitive period for the promotion of fertilization membrane formation. The thick lines show the period of periodate treatment for inducing normal membrane elevation. The eggs were transferred to periodate sea water after being inseminated in ordinary sea water in A and to ordinary sea water after being inseminated in periodate sea water in B. C shows estimated sensitive period. The under row shows the process of membrane elevation in periodate sea water.

membrane. After 1.5 minutes a faint membrane was elevated over the whole egg surface. Many spermatozoa remained still on the membrane, but some of them began to be made to detach in the sperm penetrated side. After 2 minutes the fertilization membrane shows a distinct contrast and the spermatozoa attached to the membrane decreased in number. After 5 minutes a few spermatozoa were observed on the membrane.

The periodate-sensitive period, from one minute to two minutes after insemination corresponds to the period from the initiation of the fertilization membrane formation to the completion. This suggests that the process of membrane elevation or the breakdown of the cortical granules prior to membrane elevation is promoted by periodate.

DISCUSSION

In the underripe and overripe eggs of sea urchin the irregular or tight membrane was formed after fertilization. RUNNSTRÖM and KRISZAT (1950) reported that in the underripe eggs of *Pseammechinus miliaris* fertilization membrane formation was promoted with sodium periodate. It was ascertained in the present experiment that the elevation of the fertilization membrane was improved with periodate also in *Hemicentrotus pulcherrimus*. The membrane formation of the *Hemicentrotus* eggs was remarkably promoted by the insemination in periodate sea water, while not by the periodate pretreatment.

Many researchers have reported that the fertilization- or development-inhibiting effect of jelly substances was removed with periodate in sea urchin and other invertebrate eggs (Cf. RUNNSTRÖM and KRISZAT 1950, OSANAI 1967a, b). In the *Hemicentrotus* eggs the membrane elevation was scarcely promoted by periodate pretreatment or by the removal of the jelly coat. Thus, the promotion of the membrane elevation is not due to the jelly coat.

When the eggs were transferred in periodate sea water within one minute after being inseminated in ordinary sea water, the highly elevated membrane was formed. The eggs formed also the smooth fertilization membrane when they were placed in periodate sea water for two minutes following insemination. These results suggest that periodate is effective in the period from one to two minutes after insemination. During the period the fertilization membrane was formed. Thus, it is suggested that the process of fertilization membrane formation or the breakdown of the cortical granules prior to the membrane formation was improved by periodate.

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